REMARKS/ARGUMENTS

Claims 1-8 are pending. Applicant respectfully submits that the present application overcomes all prior rejections and has been placed in condition for allowance for the reasons set forth in detail below.

I. The Invention

The presently claimed invention is directed to a plasmid containing a tightly regulated promoter operatively linked to an isolated and purified DNA sequence that encodes a peptidoglycan-associated lipoprotein (PAL) of gram-negative bacteria. Under the control of the tightly regulated promoter, the recombinant PAL is expressed in lipidated form and in yields that are higher than those expressed by a recombinant PAL that is not under the control of a tightly regulated promoter.

II. The Rejection Under 35 USC § 112, Second Paragraph

Claims 1-8 remain rejected as being indefinite for recitation of the term "tightly" in the phrase "tightly regulated promoter." The Examiner alleges that Applicant's functional definition of "tightly regulated promoters" would encompass the lac promoter, notwithstanding that the lac promoter is a leaky promoter. The Examiner concludes that the asserted definition in the specification is internally inconsistent, and therefore, one skilled in the art would be unable to ascertain the metes and bounds of a "tightly regulated promoter." (Office Action, pages 2-3).

Responsive to this rejection, Applicant encloses herein a Rule 132 declaration of Dr. Susan K. Hoiseth, who is the Associate Director of Wyeth Vaccines Research. Dr. Hoiseth defines tight, leaky and strong promoters as those terms are understood by a person of at least ordinary skill in the art.

As stated in Paragraph 6 of the Hoiseth Declaration, promoters such as the lac promoter are well known to one skilled in the art to be regulated but leaky. Specifically, a leaky promoter induces some level of protein expression in the absence of any induction.

As stated in Paragraph 7 of the Hoiseth Declaration, a tightly regulated promoter is the opposite of a leaky promoter. Unlike a leaky promoter that is always "on" even in

the uninduced state, a tightly regulated promoter refers to the degree to which a promoter can be maintained in the "off" state in the absence of induction. One can think of a leaky promoter as a very leaky faucet that cannot be shut off, and a tight promoter as a faucet that is shut off or has only a very minor leak. It is a matter of degree. In one embodiment of the present invention, described on Page 8 (lines 27-32) of the specification, the plasmid (pPX4020) was constructed to contain the arabinose inducible promoter because this promoter "is tightly regulated and almost completely inactive if no arabinose is present and some glucose is present." Guzman (1995) also refers to the arabinose promoter as a tightly regulated promoter.

As stated in Paragraph 8 of the Hoiseth Declaration, a "tightly" regulated promoter is not to be confused with a "strong" promoter. Strength refers to the maximum amount of transcription that can be achieved in the fully induced, or "on" state. A "strong" promoter would therefore be used to obtain a high level of transcription. A strong promoter can also be a leaky promoter, and a tightly regulated promoter is not necessarily a strong promoter.

The evidence presented in the Hoiseth Declaration clarifies how one of at least ordinary skill in the art would understand the subject terms as they are used in the present application. Accordingly, Applicant's definition of a tightly regulated promoter is not internally inconsistent. Although Applicant previously described what tightly regulated promoters do, as reflected in the specification at page 12 (lines 8-18), Applicant further stated at page 8 (lines 27-32) that the tightly regulated arabinose promoter is "almost completely inactive if no arabinose is present and some glucose is present." This statement conforms to what one skilled in the art considers a tightly regulated promoter.

Based on the foregoing, Applicant submits that the rejection under 35 USC 112, second paragraph, has been overcome and should therefore be withdrawn.

The Examiner also states that the Applicant did not address the issue of: "Further, it is noted that DNA can not be expressed in lipidated form and this limitation is not interpretable." Applicant asserts that the amendment to claim 1 renders this issue moot.

III. The Rejection Under 35 USC § 102(b)

Claims 1, 2 and 8 remain rejected under 35 USC 102(b) as being anticipated by Anilionis et al. (WO 90/02557) in light of Nelson et al. (*Infection and Immunity*, 56(1):128-134, 1988).[1] The Examiner maintains that the lac promoter of Anilionis fits Applicant's definition of a tightly regulated promoter. Applicant traverses the rejection.

Anticipation requires identity of invention. That is, each and every element as set forth in the claim must be disclosed in a single prior art reference, either expressly or inherently. Claim 1 as amended is directed to a plasmid constructed to contain a tightly regulated promoter operatively linked to a gene that encodes a peptidoglycan-associated lipoprotein (PAL), wherein the recombinant PAL, under the control of the tightly regulated promoter, is expressed in lipidated form and in yields that are higher than those expressed by a recombinant PAL that is not under the control of a tightly regulated promoter. Anilionis explicitly discloses in Example 8 (page 81, lines 23-25) that "[w]hen PBOMP-1 was expressed from lac or P_L promoters in *E. coli* JM103 or HB101 strain, only low levels of PBOMP-1 were expressed." Since Anilionis teaches nothing about using a tightly regulated promoter to produce large amounts of lipidated PBOMP-1, Anilionis does not teach each and every element of the claimed invention. The rejection under § 102(b) is, therefore, improper and should be withdrawn.

IV. The Rejections Under 35 USC § 103(a)

A. Claims 3-5 remain rejected as being unpatentable over Anilionis in light of Nelson, and in view of Guzman et al. (*Journal of Bacteriology*, 177(14):4121-4130, 1995). The Examiner maintains that it would have been obvious to the skilled artisan to subclone PBOMP-1 (i.e., P6) into any of the arabinose inducible vectors of Guzman because Anilionis teaches that it is desirable to use strong promoters to obtain a high level of transcription.

B. Claims 3 and 6 remain rejected as being unpatentable over Anilionis in light of Nelson, and in view of Mertens et al. (*Gene*, 164:9-15, 1995). The Examiner maintains that it would have been obvious to subclone PBOMP-1 into any of the P_{T7} containing

[1] As previously pointed out, an anticipation rejection must be based on a single reference, not a combination of references. Applicant therefore addresses only Anilionis in this rejection. Besides, Applicant himself states in the specification at page 3, line 1, that P6 is also known as PBOMP-1; therefore, Nelson is of no moment and is irrelevant for this rejection.

vectors of Mertens because Anilionis teaches that it is desirable to use strong promoters to obtain a high level of transcription, and Mertens teaches that the P_{T7} containing vectors have the *potential* to improve the expression level of other heterologous genes.

C. Claims 3, 6 and 7 remain rejected as being unpatentable over Anilionis in light of Nelson, and in view of Mertens and Novagen Inc. The Examiner maintains that it would have been obvious to subclone PBOMP-1 into the commercially available vector pET-27B of Novagen because Anilionis teaches that it is desirable to use strong promoters to obtain a high level of transcription.

Common to all of these rejections is the Examiner's reliance on Anilionis et al. (WO 90/02557). Anilionis teaches a plasmid containing a regulated promoter wherein the promoter is operatively linked to a DNA sequence encoding the PBOMP of *Haemophilus influenzae*, specifically PBOMP-1 (P6) and PBOMP-2 (P4). Anilionis also teaches fusion proteins of PBOMP-1 and PBOMP-2. In fact, the lipoprotein expressed in Anilionis is a fusion protein of the P4 and P6 outer membrane proteins of *H. influenzae.* The promoters disclosed in Anilionis include, for example, lac, trp, recA, ribosomal RNA, the P_R and P_L promoters of coliphage lambda and others including, but not limited to, lacUV5, ompF, bla, lpp and the hybrid trp-lacUV5 (tac).

Based on this disclosure, the Examiner concludes that Anilionis provides the motivation to use regulated promoters and strong promoters to obtain a high level of transcription. The Examiner misses the mark because she interprets strong promoters to be tightly regulated promoters. As pointed out in Paragraph 8 of the Hoiseth Declaration, "strong" and "tightly regulated" are not synonymous. While a strong promoter would be used to obtain a high level of transcription, a strong promoter can be a leaky promoter. In fact, the promoters used by Anilionis, such as lac and P_L, are well known as leaky promoters. Consequently, since Anilionis does not teach tightly regulated promoters to improve the expression of lipidated P6, Anilionis does not render the claimed invention obvious. Nor do any of the references combined with Anilionis fill this gap.

Nelson et al. disclose that P6 may be an important antigen in human immunity to *H. influenzae*. For this reason, Nelson et al. cloned the gene encoding P6, expressed the P6 polypeptide in *E. coli*, and sequenced the gene encoding P6 (introduction). Because of the potential problem that the *Haemophilus* promoter might fail to function in

E. coli for the expression of P6, the authors used phage and plasmid vectors containing the lac promoter in close proximity to the site of DNA insertion. However, the results of their study suggest that in *E. coli*, production of P6 is initiated by its own promoter (page 132, column 2). At the end of the article, the authors state that they will use their cloned P6 gene to further analyze the role of P6 in the human immune response. They do not address the problem of expressing lipidated P6 in meaningful quantities for commercial use.

Moreover, Nelson et al.'s plasmid contained a lac promoter, which, as explained in Paragraph 6 of the Hoiseth Declaration, is a leaky promoter. As disclosed in our specification and in Anilionis et al., leaky promoters resulted in low levels of expression of the lipidated P6 protein. So even if a person skilled in the art followed the teachings of Nelson et al., that person would have been led in a direction divergent from the path that was taken by the Applicant and would have failed. Hence, the Nelson reference "teaches away" from the claimed invention. *In re Gurley*, 31 USPQ2d 1130, 1131 (Fed. Cir. 1994) ("A reference may be said to teach away when a person of ordinary skill, upon reading the reference, would be discouraged from following the path set out in the reference, or would be led in a direction divergent from the path that was taken by the applicant.") The Federal Circuit has repeatedly recognized that proceeding contrary to the teachings in the art represents "strong evidence of unobviousness." *In re Hedges*, 783 F.2d 1038, 1041, 228 USPQ 685, 687 (Fed. Cir. 1986); *W.L. Gore & Assocs., Inc. v. Garlock, Inc.*, 721 F.2d 1540, 1552, 220 USPQ 303, 312 (Fed. Cir. 1983); *accord In re Fine*, 837 F.2d 1071, 1074, 5 USPQ2d 1596, 1599 (Fed. Cir. 1988).

Guzman et al. constructed vectors (pBAD vectors) containing the tightly regulated P_{BAD} promoter of the arabinose operon and its regulatory gene, *araC*. Guzman et al., however, do not disclose or suggest using this vector system to increase the expression of lipidated P6. At best, Guzman et al. would be viewed by one skilled in the art as an invitation to try their vector system, which is not the standard for determining obviousness.

Mertens et al. constructed a dual-promoter expression plasmid, containing both λ P_L and P_{T7} promoters, for heterologous gene expression in *E. coli*. Using these plasmids, high production levels were obtained for a number of mammalian cytokines (human tumor necrosis factor, human immune interferon, human and murine interleukins

2, murine interleukin 4 and murine fibroblast interferon). Mertens et al. therefore conclude that these plasmids have the *potential* to considerably improve the expression level of other heterologous genes. Again, having the *potential* to improve expression levels does not provide a reasonable expectation of success. It merely invites the skilled artisan to experiment. Mertens et al. even acknowledge that despite the wide experience in the field, high-level expression is often a result of trial and error.

Novagen Inc. supplies the commercially available expression vector pET27b, which has the T7 promoter. In view of Mertens et al. teaching that the P_{T7} promoter is a strong promoter that can be tightly regulated, the Examiner concludes that it would have been obvious to subclone P6 into pET27b because Anilionis teaches that it is desirable to use strong promoters to obtain a high level of transcription. Anilionis, however, does not teach or suggest that using a tightly regulated promoter will solve the problem of low-level expression of lipidated P6. Nor does the Examiner's combination of references show or suggest the properties and results of the claimed invention or suggest the claimed combination as a solution for improving the expression of lipidated P6. Consequently, they cannot be successfully relied upon for an obviousness rejection. *In re Wright*, 6 USPQ2d 1959 (Fed. Cir. 1988).

Moreover, the Examiner cannot ignore that many laboratories tried and failed to express large amounts of lipidated P6, and they all had the same references available to the Examiner to teach them how to do it. (See specification, pages 3 and 4, and amendment dated February 25, 2004.) They failed, plain and simple. Applicant figured out what combination would work, and it is Applicant's plasmid construct that is responsible for increased expressions levels of lipidated P6.

In view of the foregoing, Applicant respectfully submits that the rejections of the claims as amended are improper and should be withdrawn.

Applicant respectfully requests that a timely Notice of Allowance be issued in this case.

Respectfully submitted,

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